CLASSIFICATION OF NON-CODING RNA USING GRAPH REPRESENTATIONS OF SECONDARY STRUCTURE

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Some genes produce transcripts that function directly in regulatory, catalytic, or structural roles in the cell. These non-coding RNAs are prevalent in all living organisms, and methods that aid the understanding of their functional roles are essential. RNA secondary structure, the pattern of base-pairing, contains the critical information for determining the three dimensional structure and function of the molecule. In this work we examine whether the basic geometric and topological properties of secondary structure are sufficient to distinguish between RNA families in a learning framework. First, we augment the dual graph representation of RNA secondary structure proposed by [1] with biologically meaningful labels. We define a similarity measure directly on these graph objects using the recently developed marginalized kernels of [2]. Using this learning method, we trained Support Vector Machine classifiers to distinguish known RNA families from random RNAs. For 22 of the 25 families tested, the classifier achieved better than 70% accuracy, with much higher accuracy rates for some families. A *one vs. all* multi-class scheme also showed limited success. From these initial learning experiments, we suggest that the *labeled dual graph* representation, together with kernel machine methods, has potential for use in automated classification of uncharacterized RNA molecules or efficient genome-wide screens for RNA molecules from existing families.

1. Introduction

Non-coding RNA (ncRNA) molecules are those RNAs that do not encode proteins, but instead serve some other function in the cell [3]. They play a variety of critical roles [4] and are ubiquitous in all kingdoms of life. RNA

function is uniquely determined by the three dimensional structure of the molecule. To reach its functional form, a single stranded RNA molecule folds back on itself – driven by GC/AU/GU base-pairing and stacking interactions – to form short helices and various single stranded loop regions that define its secondary structure [5]. Some RNAs require metals or proteins to chaperone the folding process, but for the most part, the final three dimensional structure, and hence the functional role, is fully determined by the secondary structure [6]. This suggests that development of computational tools based on RNA secondary structure is essential for discovery and classification of new non-coding RNAs.

A variety of computational methods have used the secondary structure of RNA molecules to search and categorize ncRNAs, but many of these methods are limited in their use of secondary structure. Regular-expression-like pattern matching algorithms have been used to scan genome sequences for regions that fold into the canonical structures of specific families [7]. However, they are designed to stringent configurations of secondary structure elements, and therefore perform poorly on families with variations in folding. Pair Stochastic Context Free Grammars (P-SCFG) look for evidence of secondary structure conservation by modeling covariance of mutations from related genomes [8] – but determining an approrpriate grammar is a non-trivial problem [9] Some discriminative classifiers use secondary structure stability as an input feature to distinguish non-coding RNAs from intergenic sequence [10], but they ignore important topological information. On the other hand, methods that use computable representations of secondary structure, such as trees and graphs, have been restricted to categorization and enumeration of gross topological features [11, 1].

Here we present a kernel-based machine learning method for classifying RNA families that avoids some of these limitations by learning directly from a graphical representation of secondary structure. This discriminative method does not require the adaptation of any parameters or training of cumbersome generative models, yet it captures some of the topological relationships of RNA secondary structures. First, we define an appropriate representation of RNA secondary structure by extending the RNA dual graph [1] representation with a biologically relevant labeling scheme. Second, we define a similarity measure between RNA secondary structures by applying a recently developed marginalized kernel [2] to compare RNA molecules encoded as labeled dual graphs. Third, we test the usefulness of this representation for learning the topological characteristics of RNA secondary structure by training Support Vector Machines [12] on appropriate

datasets.

2. An Algorithm for Classification Based on Secondary Structure Topology

Classification of RNA secondary structures with Support Vector Machines (SVM) requires both a representation that captures the secondary structure and a kernel function that provides a reasonable similarity measure for the chosen representation. Below we describe a graph representation that captures some topological properties of the secondary structure of RNAs, as well as a method for applying kernel functions to the graph representations.

2.1. *Labeled Dual Graphs*

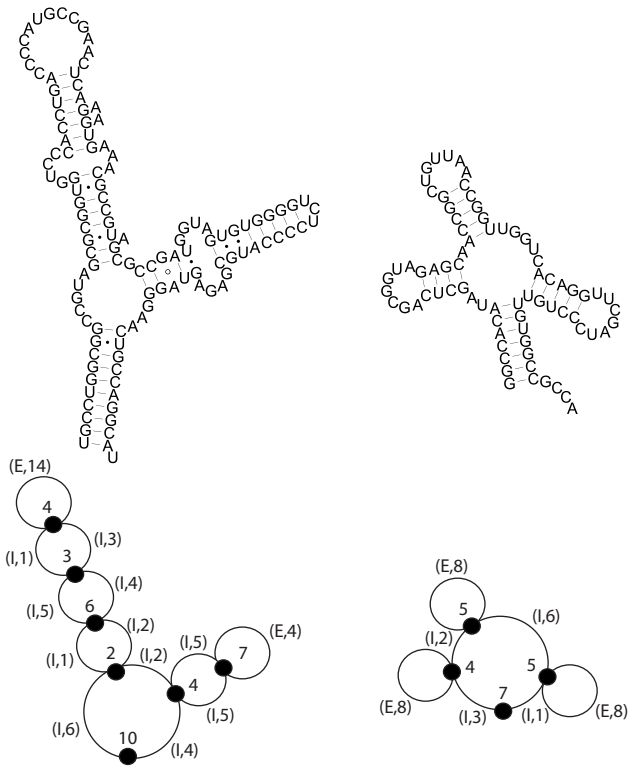
Given a secondary structure of an RNA molecule, we want to construct a graph that captures essential properties of the structure. We begin by converting the RNA secondary structure to a dual graph [1]. In this representation, helical regions of the RNA are represented as vertices of the graph, while single RNA strands that connect the helical regions are edges. Thus, internal loops, bulges, and multi-loops become edges that connect vertices (helices adjacent to the loops), and external loops become edges from a vertex to itself.

We then augment the graph representation by adding labels that correspond to the length and type of secondary structure elements. The resulting *labeled dual graphs* (LDGs) are comprised of vertices labeled according to the number of nucleotide-pairs in the helical region they represent, and edges labeled according to the length (in number of nucleotides) and type (internal/external) of the loop they represent. See Figure 1 for an illustration of labeled dual graphs.

2.2. *Marginalized Kernels for Labeled Dual Graphs*

In order to use an SVM classifier on graph objects, we need a kernel function to define a similarity between two labeled dual graphs. Several kernels for graph objects have been proposed [13, 2]; here we use the recently developed *marginalized kernel* for labeled graphs [2] because it is relatively simple to implement, computationally efficient, and yielded promising results. Intuitively, this kernel function computes a similarity measure between two arbitrary labeled graphs by comparing the label sequences produced by taking random walks on each of the two graphs; the more similar the sets of label sequences, the higher the similarity score for the pair of graphs.

A



B

$L=1$	$\{4\},\{3\},\{6\},\{2\},\{10\},\{4\},\{7\},$	$\{5\},\{4\},\{7\},\{5\}$
$L=2$	$\{4,(\mathrm{E},14),4\},\{4,(\mathrm{I},1),3\},\ldots$	$\{7,(\mathrm{I},3),4\},\{5,(\mathrm{I},6),5\},\ldots$
$L=3$	$\{4,(\mathrm{I},5),7,(\mathrm{E},4),7\},\ldots$	$\{7,(\mathrm{I},3),4,(\mathrm{E},8),4\},\ldots$
$L=4$	$\{7,(\mathrm{I},1),5,(\mathrm{E},7),5,(\mathrm{I},4),5\},\ldots$	$\{7,(\mathrm{I},5),7,(\mathrm{E},55),7,(\mathrm{I},7),8\},\ldots$
$\vdots$		$\vdots$

C

$$K_z = K_v(4,7)K_e(5,3)K_v(7,4)K_e(4,8)K_v(7,4)$$
$$= exp(-(\log\frac{4}{7})^2 - (\log\frac{5}{3})^2 - (\log\frac{7}{4})^2 - (\log\frac{4}{8})^2 - (\log\frac{7}{4})^2)$$

Figure 1. *A.* Secondary structure diagram and *labeled dual graph* representation of 5S rRNA (left) and tRNA (right) molecules. The numbers and ordered pairs are the vertex (helix) and edge (loop) labels, respectively. The labels E and I are used to distinguish external from internal loops. *B.* A subset of label sequences generated by taking random walks on the two graphs. Here L refers to the length of the path. *C.* An example of the label sequence kernel and its output, as it is applied to a pair of label sequences. We consider the two paths with $L = 3$.

The computation of the kernel function between two graphs G and G' proceeds as follows. First, generate a random walk h on graph G and a walk h' on graph G', according to some defined probability of transitioning from vertex to vertex. Each walk produces a sequence of vertex and edge labels, $z = \{v_1, e_{12}, v_2, e_{23}, v_3, \ldots\}$ and $z' = \{v'_1, e'_{12}, v'_2, e'_{23}, v'_3, \ldots\}$. Next, define the label sequence kernel $K_z(z, z')$ as the product of the vertex label kernels $K_v(v, v')$ and the edge label kernels $K_e(e, e')$ over the sequence of labels,

$$K_z(z, z') = K_v(v_1, v'_1)K_e(e_{12}, e'_{12})K_v(v_2, v'_2)\ldots . \quad (1)$$

If the two walks are of different lengths, we assume the label sequence kernel is 0. Now that a similarity measure $K_z(z, z')$ is defined for each pair of walks, the value of the full graph kernel $K(G, G')$ is computed as the expected value of $K_z(z, z')$ over all possible walks h and h', weighted by the probability of generating the walks,

$$K(G, G') = \langle K_z(z, z')\rangle_{h,h'} . \quad (2)$$

The probability of taking a random walk on a graph, $p(h, h')$ depends on the probability of starting at a particular vertex and transitioning to subsequent vertices. We assumed a uniform starting probability over all vertices, a uniform probability of transitioning from a vertex to one of its neighbors, and a constant probability (0.1) of terminating the walk after any step.

Finally, we need to specify the edge and the vertex kernel functions, $K_e(.,.)$, $K_v(.,.)$. These should reflect the similarities in RNA structural motifs – similar helices should produce high similarity scores, as should comparable loops. The choice of biophysical parameters that can serve as the basis for similarity comparisons is large – base composition, sequence or structural alignment, feature lengths, among others. As a first step we chose edge and vertex kernels that reflect the most basic structural parameters: the number of nucleotides that comprise a secondary structure motif. The vertices and edges of the dual graphs are labeled with these distances, and the vertex and edge kernels are defined as the Gaussian distance on the log-ratio of the two labels (lengths). The vertex kernel is thus defined as

$$K_v(v_i, v_j) = exp(-\lambda_{ij}^2) , \quad (3)$$

where $\lambda_{ij} = log(v_i/v_j)$. For two edges of the same type of loop (internal or external), the edge kernel is similarly defined,

$$K_e(e_{ij}, e_{kl}) = exp(-\lambda_{ij,kl}^2) , \quad (4)$$

and for edges of different types, the edge kernel is 0. See Figure 1b for an illustrative example.

Effectively, two labeled dual graphs are considered similar when the two sets of all possible walks on each graph are similar. The similarity between individual walks is calculated as a product of simple functions defined on their constituent labels. Thus, if all the vertex and edge labels in the two walks match up, the output of the kernel function on the two walks will be high; and if many of the walks on the two graphs are similar, the kernel function will return a high value (with $\max K(G, G') = 1$) for the two graph objects. Hence this computation captures some topological relationships between structural elements of RNA secondary structure.

3. Methods

We performed two sets of experiments to test the ability of the classifier to learn RNA secondary structure and predict RNA family labels. First we trained SVM classifiers to distinguish non-coding RNAs from random RNAs with similar di-nucleotide composition. We also trained a system of multi-class SVMs to determine the family RNA sequences.

Single family classification was tested on a number of RNA families from the RFAM database [14] (see the Results section for the list of tested RFAM families). When possible, we trained and tested the classifier on 500 RNA sequences, randomly selected from all RNAs in the family. However, some RFAM families contained fewer sequences, in which case all were used for classification. The negative data set was constructed by shuffling the nucleotide sequences of the positive data set while preserving the di-nucleotide frequencies [15], which destroys characteristic secondary structure but produces random RNAs with sequence statistics similar to real RNA.

RNA sequences were converted to secondary structures with the Vienna RNA [16] folding prediction package, then converted to labeled dual graphs as described above. We implemented the kernel computation using an iterative method described in [2]; one thousand kernel computations took between 2 and 40 seconds on a desktop machine (2GHz Athlon), depending on the average complexity of the secondary structure. SVM classification was performed with 10 fold cross validation, with the precision parameter set to 10000. We assessed classifier performance with sensitivity ($TP/(TP+FN)$) and specificity ($TN/(TN+FP)$) measures and by computing the area under ROC curves (A_{ROC}) [17].

We also trained a multiclass classifier on nine large RFAM families us-

ing the *one vs. all* method, a simple and common approach to multiclass classification [18]. A separate classifier was trained to distinguish each family from the other eight. During classification, an RNA sequence was tested against each of the nine classifiers, and a family label was assigned according to the classifier that produced the highest decision value. A number of related families with few RNA sequences were grouped together for training and analysis (see Results for details). Performance was assessed with the generalized *class precision* and *class recall* measures [19]. For each classifier, the class sensitivity (Q^D) represents the percentage of samples correctly predicted relative to the total number of samples in that family, while the class specificity (Q^M) captures the number of samples correctly predicted relative to the total number of samples predicted to be in that family.

4. Results

4.1. *Single Family SVM*

Figure 2 shows the results of SVM classifiers trained on datasets derived from individual RFAM families. For sufficient training data we used families with 50 or more sequences. The generation of negative training data is described in the previous section. The classifiers showed good performance for a large number of families, with $A_{ROC} > 0.7$ for 22 of 25 families tested. This suggests that the learning method is useful for learning a variety of secondary structure topologies. A notable result is the good classifier performance on several riboswitch and microRNA families, two particularly exciting non-coding RNA classes that have recently been shown to be involved in novel mechanisms for regulating gene expression.

4.2. *Multi-class SVM*

Table 1 shows the cross validation results of the *one vs. all* multi-class SVM trained on nine RFAM families. The MICRO and RNASE groups represent aggregates of functionally related individual RFAM families (see the caption for details). Again, classifier sensitivity and specificity were good over a range of families, although specificity clearly degraded for RNA families with larger molecules and possibly more complicated secondary structures. In these instances, it is possible that shorter walks pick up spurious similarities.

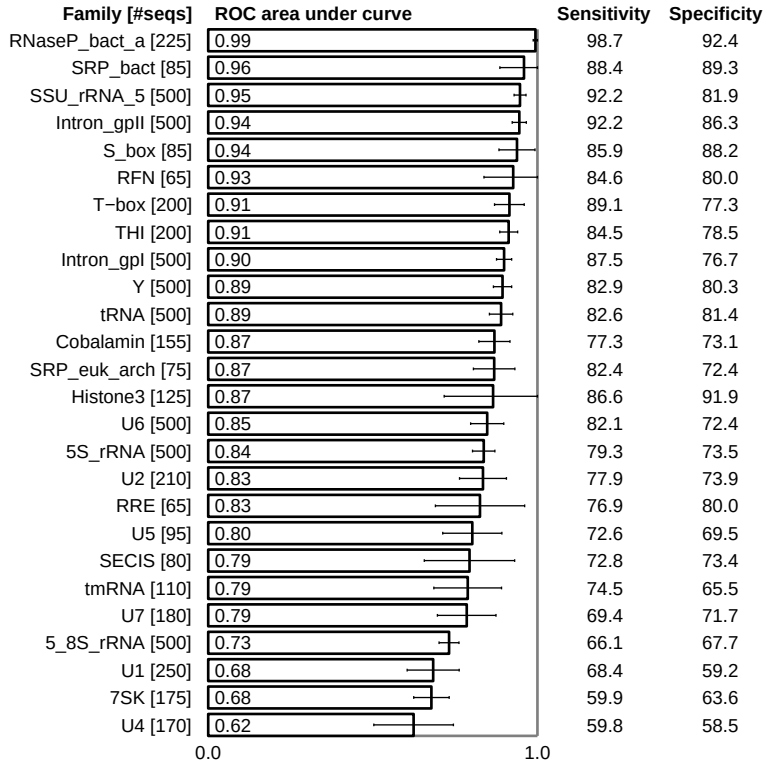


Figure 2. Performance of SVM classifiers trained on single RFAM families vs. shuffled sequences with the same di-nucleotide composition. Area under the ROC curve (A_{ROC}) is computed as the mean of the areas for each ROC curve of the 10 cross validation trials; error bars are standard deviation of A_{ROC}.

5. Discussion

The method presented here was able to learn to distinguish a number of non-coding RNA families; however, it is worth highlighting a few factors that may have adversely impacted its performance. First, the algorithm relies on predicted secondary structures as input, and is therefore sensitive to incorrectly predicted structures. As an example, training and testing a classifier on tRNAs for which correct folding was manually verified increased the accuracy from 89% to 98%. On the other hand, because the kernel computation considers local paths over the entire structures, parts of the molecule that are correctly folded will still contribute to the correct computation of the kernel, even if some parts of the molecule are mis-folded.

Table 1. Contingency table showing results for 10 fold cross validation of *one vs. all* multi-class SVM. For each RNA family (table row), the number of RNAs classified as a certain family appears in the respective column. Q^D and Q^M refer to generalized sensitivity and specificity, respectively. If z_{ij} is an element in the contingency table, then $Q_i^D = \frac{z_{ii}}{\sum_j z_{ij}}$ and $Q_i^M = \frac{zii}{\sum_j z_{ji}}$. Several functionally related small RFAM families were grouped together to form aggregate families, **MICRO**: *let-7, lin-4, mir-1, mir-10, mir-101, mir-103, mir-124, mir-130, mir-135, mir-148, mir-156, mir-16, mir-160, mir-166, mir-17, mir-181, mir-19, mir-192, mir-194, mir-196, mir-199, mir-2, mir-218, mir-219, mir-24, mir-26, mir-29, mir-30, mir-46, mir-6, mir-7, mir-8, mir-9*; and **RNASE**: *RNaseP_bact_a, RNaseP_bact_b, RNaseP_nuc, RNase_MRP*, and these were trained and tested as single classes.

	Histone3	Intron_gpI	Intron_gpII	MICRO	RNASE	SSU_rRNA_5	tRNA	U6	Y	Q^D
Histone3	123	0	0	0	0	0	1	3	0	**.97**
Intron_gpI	0	355	82	1	18	33	1	5	5	**0.71**
Intron_gpII	0	27	443	0	5	7	6	9	3	**0.89**
MICRO	0	3	2	165	0	0	1	1	8	**0.92**
RNASE	0	26	5	1	251	52	0	3	3	**0.74**
SSU_rRNA	0	17	0	0	5	474	0	1	3	**0.95**
tRNA	0	16	8	4	8	27	370	33	26	**0.75**
U6	0	17	4	0	4	25	10	409	31	**0.82**
Y	0	32	3	4	11	31	14	30	375	**0.75**
$\mathbf{Q^M}$	**1.0**	**0.72**	**0.81**	**0.94**	**0.83**	**0.73**	**0.92**	**0.83**	**0.83**	

More accurate folding algorithms should improve the performance of this classifier. Also, existing secondary structure prediction algorithms are capable of predicting multiple suboptimal secondary structures. One possibility for minimizing the effect of incorrectly predicted secondary structures is by utilizing sets of dual graphs corresponding to these suboptimal structures.

As a representation of RNA secondary structure, the current formulation of labeled dual graphs does lose some of the structural information: the natural 5'-3' directionality of the molecule, the lengths of free 5' and 3' strands, as well as more complex topological features such as chirality or relative position of helical regions. Much of this information could be included with natural extensions to the labeling scheme, together with appropriate modifications to the kernel computation.

6. Conclusion

We have presented a novel, simple, and computationally efficient approach for learning RNA secondary structures. It uses graph representations of folded RNA structures and kernels defined on graph objects to train SVM

classifiers. Applied to non-coding RNAs from the RFAM database, the method gave promising results. It could distinguish many families from random RNA sequences with identical di-nucleotide composition, and showed some ability to differentiate one family from another. Because this conceptually simple approach produced relatively accurate classifiers, and because no other automated discriminative method for classification or discovery of ncRNAs exists, we believe there is great potential for extending this method or combining it with other techniques. Specific applications could include automated class-discovery of uncharacterized RNA molecules and computationally efficient heuristic filters in conjunction with other methods for RNA family prediction.

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